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(54) Title: ENZYMATIC PREPARATION OF GALACTOSE AND/OR GLUCONIC ACID FROM DI- OR POLYSACCHARIDES

(57) Abstract

A process for the large scale purification of the hexose sugar galactose. The process comprises hydrolysing a galactose-containing disaccharide or polysaccharide into its constituent monomers; non-galactose sugar monomers are then converted into non-sugars by the addition of enzymes selective for these monomers. The galactose may then be removed using known purification techniques.

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ENZYMATIC PREPARATION OF GALACTOSE AND/OR GLUCONIC ACID FROM DI- OR POLYSACCHARIDES

Field of the Invention

This invention relates to the large scale production of the hexose sugar galactose, and optionally also gluconic acid, having a high level of purity.

Background of the Invention

Galactose is a sugar which, although occurring naturally in relatively large quantities, is not used in nature as a storage sugar to the same extent as glucose or sucrose. The production of galactose is therefore not simply a means of extraction. Rather, it needs to be prepared from other naturally-occurring disaccharides or other large polymers. Galactose may be used in the pharmaceutical industry and other industries as an excipient or as a precursor for chemical and pharmaceutical synthesis. Although galactose can be prepared in a relatively pure form on a small scale, large scale extraction has been beset by difficulties. In particular, significant amounts of other materials are present in the final preparation, and these contaminants or by-products are often extremely difficult to separate from the galactose. High levels of contamination render the product largely unsuitable as a pharmaceutical material.

Summary of the Invention

The present invention provides a novel approach to the production of galactose using cheap raw materials. The process comprises hydrolysing a galactose-containing disaccharide or polysaccharide into its constituent sugar monomers; non-galactose sugar monomers are then converted into non-sugars by the addition of enzymes selective for these monomers. The galactose may then be removed easily using known purification techniques, and can have pharmaceutical grade purity.

Description of the Invention

Typically, any galactose-containing disaccharide or polysaccharide molecule may be used as the starting material. Preferably, the starting material contains a

high level of galactose monomer units. A suitable disaccharide is lactose (milk sugar) which is available cheaply and in large quantities as a by-product of the dairy industry.

The particular starting material used will determine which enzyme(s) are to be used in the later steps of converting the non-galactose monomers into non-sugar molecules. For example, if lactose is used as the starting material, the products of hydrolysis will be galactose and glucose. The glucose may then undergo further reaction by introducing the enzyme glucose oxidase which converts glucose into gluconic acid.

It may sometimes be desirable to introduce additional enzymes which utilise some of the by-products of these reactions. For example, the conversion of glucose into gluconic acid using glucose oxidase produces hydrogen peroxide as a by-product. This may be removed by converting the peroxide to water using the enzyme catalase.

Suitable process conditions will be apparent to the skilled person, and optimum conditions can be determined by reasonable trial. Typically, the most important parameters will be the concentration of starting materials and the efficiency of the various enzymes. For example, if lactose is used as the starting material, hydrolysis may be achieved using a high activity β-1,4-galactosidase. The concentration of lactose may be varied and will typically be within 10-65%. Preferably, the lactose will be present at a concentration of about 20%. Other parameters may be adjusted to achieve the optimum conditions, e.g. reaction time and temperature of the reaction.

The reactions may be carried out in a batch process with all the reactants present together, or may be carried out by adding the reactants sequentially after completion of each preceding reaction. The reaction will typically occur in a stirred reaction vessel. Oxygen can be introduced into the reaction by adding air, oxygen enriched air or pure oxygen to the tank.

Separation and purification of the galactose may be carried out easily using conventional techniques. For example, the galactose may be separated using differential purification, affinity purification, separation by molecular weight, by ionic charge (ionic exchange) or by using affinity columns.

The final stage in the process is the removal of water which may be achieved by using any conventional method, e.g. vacuum evaporation or reverse osmosis (RO). If a dry powder is required, this may be achieved by spray-drying a concentrated galactose solution.

The following Example illustrates the invention.

Example

Stage 1: Hydrolysis of Lactose to Glucose/Galactose (50:50)

100 kg lactose (Pharmaceutical Grade) was dissolved in 400 kg water (RO quality) in an agitated stainless steel jacketed processing vessel, connected to a controlled hot water set and cooling water circuit. The solution is maintained at 50±3°C using the hot water set. The pH of this solution was maintained between 4.0 and 6.0. β-galactosidase with an activity of 40,000 U/g was added to the solution, and samples taken for analysis at 20-minute intervals. Analysis was carried out using HPLC to determine lactose, and glucose/galactose levels. The reaction was allowed to run for 300 min, until the desired percentage conversion had been achieved (>98%).

Stage 2: Enzymatic Oxidation of Glucose

The glucose/galactose solution produced in Stage 1 was then cooled to 40°C using a cooling water circuit on the process vessel jacket. The pH of the solution was adjusted to 7 using calcium carbonate. An enzyme mixture of glucose oxidase/catalase with activities of 10,000/100,000 U/g respectively was added to the solution. Oxygen was injected into the solution using a bubble diffuser at the rate of 10 L/min with continuous agitation. Samples were withdrawn at 90-minute intervals and analysed for glucose and galactose content using Boehringer enzyme analysis

kits. The reaction was allowed to proceed until conversion of the glucose had reached >99%, as shown in Tables 1A and 1B.

The analysis comprised the measurement of either NADPH or NADH, formed in a reaction utilising D-glucose or D-galactose, respectively. Measurements were carried out by spectrophotometry at 340nm. "Factor" refers to the standard calculation for the concentration of a sample, taking into consideration any dilution factor.

Table 1A

Galactose				
Time (Hours)	Blank 340nm	Absorption 340nm	Factor (g/l)	Concentration (g/l)
0	0.031	0.638	94.37	57.28
4.5	0.031	0.638	94.37	57.28
9	0.014	0.616	94.37	56.81

Table 1B

Glucose				
Time (Hours)	Blank 340nm	Absorption 340nm	Factor (g/l)	Concentration (g/l)
0	0.135	0.745	93.51	57.04
1.5	0.143	0.587	93.51	41.52
3	0.041	0.444	93.51	37.68
4.5	0.041	0.287	93.51	23.00
6	0.041	0.129	93.51	8.23
7.5	0.041	0.878	9.35	7.83
9	0.041	0.601	9.35	5.24
10.5	0.041	0.328	9.35	2.68
12	0.358	1.358	0.94	0.94

The reaction can be considered as:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-gluconic acid} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

After the reaction was complete, the solution was cooled using the cooling water circuit, and filtered to remove the precipitated calcium gluconate and any remaining calcium carbonate using a pressure leaf filter.

The solution was then heated to 85°C for 30 minutes to precipitate the enzymes. The solution was again filtered through the pressure leaf filter to remove the enzyme. The solution was then cooled before final processing.

Stage 3: Removal of Calcium Ions

The solution was passed through a cationic resin bed to remove any remaining calcium ions. The pH of the resulting solution fell to 2.2 as the calcium gluconate was converted to gluconic acid. A rise in pH indicated exhaustion of the resin, which was regenerated with dilute hydrochloric acid solution. The resin was finally washed with RO water. The process was then continued until all of the solution had been processed.

Stage 4: Removal of Gluconate

The solution from Stage 3 was passed through an anionic resin suitable for the removal of gluconate ions. The resulting solution contained only free galactose. A fall in the pH indicated exhaustion of the resin, which was regenerated using dilute sodium hydroxide solution. Finally, the resin was washed, again using RO water. The process continued until all the solution had been processed.

Stage 5: Final Processing

The dilute galactose solution was evaporated to various levels, depending on application. In addition, there was the optional final step of spray-drying to a powder.

CLAIMS

1. A process for the production of galactose from galactose-containing disaccharide or polysaccharide molecules, comprising the steps of:

(i) reacting the disaccharide or polysaccharide molecules under conditions sufficient for hydrolysis to occur, to form galactose and other saccharide molecules;

(ii) reacting the product of step (i) with an enzyme selective for the other saccharide molecules; and

(iii) separating the galactose from the products of step (ii).

2. A process according to claim 1, wherein the disaccharide is lactose.

3. A process according to claim 1 or claim 2, wherein the enzyme of step (i) is β-galactosidase.

4. A process according to any preceding claim, wherein the galactose is separated by differential precipitation.

5. A process according to any preceding claim, wherein the enzyme of step (ii) is glucose oxidase and the non-sugar is gluconic acid.

6. A process according to claim 5, wherein step (ii) further comprises the enzyme catalase.

7. A process according to claim 5 or claim 6, wherein the gluconic acid is separated by ion-exchange chromatography.